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Teniposide Induces Nuclear but not Mitochondrial DNA Degradation[1]

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ABSTRACT

Teniposide [4′-demethylepipophyllotoxin-4-(4,6-*O*-thenylidene-β-D-glucopyranoside) (VM-26)] is a cancer chemotherapeutic drug with a high target specificity for DNA topoisomerase II. This agent induces repairable protein-bridged double-strand DNA breaks, which have been correlated with cytotoxicity, but high concentrations of VM-26 also induce irreversible DNA degradation and apoptotic cell death. It is not known whether this degradation occurs uniformly throughout the genome or in a gene-specific manner. To answer this question, DNA was isolated from HL-60 promyelocytic leukemia cells exposed to 5 μM VM-26 for varying periods of up to 12 h. Nucleosomal "ladders" on 2.0% agarose gels stained with ethidium bromide were detectable after 3 h of exposure, indicative of apoptosis. Gene-specific DNA degradation was investigated by Southern blot analysis. The genes for 18S rRNA and glucose-6-phosphate dehydrogenase were representatives of constitutively expressed (*i.e.*, "housekeeping") genes. The proto-oncogenes c-*myc*, c-Ha-*ras*, and *bcl-2* were examined as examples of other transcriptionally active genes, while transcriptionally inactive genes in HL-60 cells were studied by probing for the immunoglobulin heavy chain joining region and λ light chain constant region genes. The rates of DNA degradation, and its extent after 12 h, were similar for all nuclear genes studied. However, there was striking resistance of mitochondrial DNA to endonucleolytic degradation. These data demonstrate that VM-26 can elicit a widespread degradative process which affects nuclear but not mitochondrial DNA.

INTRODUCTION

Chemotherapeutic drugs teniposide (VM-26[3]) and its less potent analogue etoposide (VP-16) inhibit the activity of topo II (1, 2). Interaction of either agent with this enzyme has the potential to produce several DNA lesions. Of these, protein-bridged double-strand DNA breaks resulting from stabilization of the cleavable complex have been proposed to account for the cytotoxicity of topo II inhibitors (3–5). This complex is normally formed by the covalent linkage of each of the two homologous topo II subunits to the 5′-phosphoryl ends of the DNA strand following cleavage by this enzyme, but its stabilization prevents religation of the DNA backbone after passage of another strand of DNA through the incision. These breaks become apparent when cells are lysed in the presence of a detergent (*e.g.*, SDS) (3, 5, 6). It is postulated that interaction of two drug molecules with topo II (*i.e.*, one molecule/subunit) is required for complete inhibition and subsequent generation of a double-strand break, but single-strand DNA breaks also occur, possibly due to the inhibition of only one subunit (3). Other possible mechanisms of cytotoxicity include the deletion, insertion, and/or recombination of DNA sequences resulting from exchange of subunits between two transiently inhibited topo II molecules (7).

Many neoplastic cells possessing a high proliferative potential are sensitive to VM-26 and VP-16. These include cell lines derived from various leukemias, lymphomas, and lung carcinomas (8–11). The correlation of double-strand DNA breaks with cytotoxicity in such cell lines has led to studies of the gene-specific induction of breaks. Several groups have demonstrated the presence of topo II cleavage sites within activated oncogenes when the activity of this enzyme is inhibited (12, 13). For instance, exposure of the small cell lung carcinoma cell line NCI-N417 to VM-26, VP-16, or the DNA intercalator 4′-(9-acridinylamino)methanesulfon-*m*-anisidide results in site-specific cleavage of the amplified and transcriptionally active c-*myc* oncogene, while the unexpressed c-*mos* proto-oncogene remains intact (14, 15). However, it is not clear how the topo II-mediated DNA breaks lead to cell death.

In addition to the induction of double-strand DNA breaks, exposure of HL-60 promyelocytic leukemia cells to VM-26, VP-16, and other chemotherapeutic agents induces the degradation of DNA into fragments of 180-base pair multiples that have a ladder-like appearance when this DNA is electrophoresed on agarose gels and visualized by ethidium bromide staining (16, 17). This is a biochemical hallmark of apoptosis, also referred to as programmed cell death (18, 19). During this process, a cellular Ca^{2+}/Mg^{2+}-dependent endonuclease is activated and digests internucleosomal DNA (20–22). Nucleosomal DNA is protected from degradation by the histone octamer, and this generates the DNA "ladders" seen in agarose gels. Morphological criteria of apoptosis include the condensation of nuclear contents, with subsequent fragmentation of nuclei into membrane-bound apoptotic bodies, and the persistence of cell membrane integrity, which can be verified by the exclusion of the vital dye trypan blue (19).

The pathway of apoptosis has been shown to be irreversible and occurs in a variety of settings, including embryological development, differentiation, and glucocorticoid-induced death of thymocytes (23–25). While the importance of apoptosis as the mode of cell death that results from exposure to cancer chemotherapeutic agents is becoming increasingly more apparent, many details remain to be clarified. In this report, we demonstrate that (*a*) the rate and extent of degradation of a nuclear gene are not influenced by the transcriptional activity of the gene and (*b*) mtDNA remains intact during VM-26-induced apoptosis of HL-60 cells, at the time when a widespread nuclear DNA-degradative process is occurring.

MATERIALS AND METHODS

Cell Lines and Culture. HL-60 (G_1) was subcloned from an early passage of HL-60 human promyelocytic leukemia cells and are highly sensitive to agents that induce monocytic differentiation. These cells were grown in suspension in RPMI 1640 medium (Mediatech, Washington, DC) supplemented with 10% fetal calf serum (Hyclone Labo-

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[3] The abbreviations used are: VM-26, 4′-demethylepipophyllotoxin-4-(4,6-*O*-thenylidene-β-D-glucopyranoside); CHX, cycloheximide; C_λ, immunoglobulin λ light chain constant region; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; G6PD, glucose-6-phosphate dehydrogenase; HBSS, Hanks' balanced salt solution; J_H, immunoglobulin heavy chain joining region; mtDNA, mitochondrial DNA; SDS, sodium dodecyl sulfate; topo II, DNA topoisomerase II; VP-16, VP-16-213, 4′-demethylepipodophyllotoxin-9-(4,6-*O*-ethylidene-β-D-glucopyranoside); cDNA, complementary DNA; SSC, standard saline citrate.

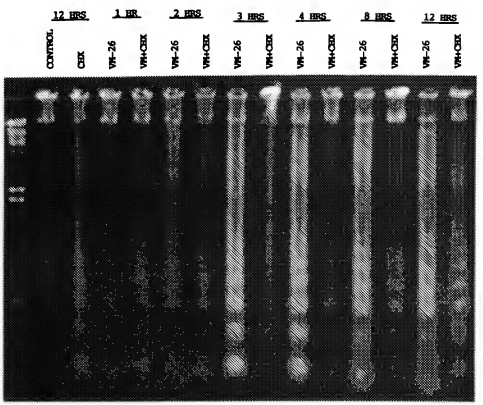

Fig. 1. Time course of VM-26-induced DNA degradation and its inhibition by CHX. High molecular weight DNA was isolated from untreated HL-60 (G_1) cells (*CONTROL*) and those treated with VM-26 (5 μM) alone, CHX (20 μg/ml) alone, or the combination (*VM+CHX*). The DNA was electrophoresed through 2.0% agarose and subsequently stained with 0.5 μg/ml ethidium bromide.

ratories, Inc., Logan, UT), 2 mM glutamine (Mediatech), 50 IU/ml penicillin, and 50 μg/ml streptomycin (Mediatech), in a 37°C incubator with a humidified environment of 5% CO_2 in air. For experiments in which calcium-sufficient and -deficient conditions were compared, cells were cultured in HBSS, with or without calcium chloride, supplemented with 0.5% bovine serum albumin (Pentax Fraction V) and 10 mM *N'*-[2-hydroxyethyl]piperazine-*N'*-2-ethanesulfonic acid. EGTA (8 mM) was added to the calcium-deficient HBSS to chelate the remaining calcium ions (26).

Chemicals and Treatments. Teniposide (VM-26) was the generous gift of Bristol-Meyers Oncology (Wallingford, CT). It was obtained as a 10 mg/ml pharmaceutical preparation or in powder form, from which a stock solution of the same concentration was prepared in dimethyl sulfoxide. The former was stored at 4°C and the latter at −20°C. Before use, a 5 mM working solution was prepared in RPMI 1640 medium. A 10 mg/ml stock solution of cycloheximide (Sigma) was prepared in Milli-Q purified water, filter sterilized, and stored at −20°C.

DNA Probes. The plasmid pRyc 7.4 (obtained from Dr. G. Rovera, Wistar Institute, Philadelphia, PA) contains a 1.2-kilobase cDNA insert corresponding to exons 2 and 3 of the *c-myc* proto-oncogene cloned into the *Pst*I site of pBR322. Probes for the human c-Ha-*ras* and G6PD genes were obtained from the American Type Culture Collection (Rockville, MD). The pUC EJ 6.6 (pEJ6.6) plasmid contains a 6.6-kilobase DNA fragment containing an activated c-Ha-*ras* oncogene isolated from the human bladder carcinoma EJ cell line and cloned into pUC13. Plasmid pGD-P-25A is a 1.5-kilobase fragment of the G6PD gene inserted into the *Eco*RI site of pUC18. The cDNA probe for the 18S rRNA gene was obtained from R. Guntaka (University of Missouri, at Columbia). The C_λ probe (generously provided by Dr. K. Raska, New Jersey Medical School) was a construct composed of a 3.5-kilobase genomic fragment inserted into pBR322. The J_H probe was purchased from Oncor (Gaithersburg, MD). The mitochondrial DNA probe p72 has been described by us previously (27).

DNA Isolation. High molecular weight DNA was isolated from samples of 10^8 cells, by a slight modification of established methods (28, 29). Briefly, cells were lysed by incubation at 50°C for 12 h, in a digestion buffer containing 100 mM NaCl, 10 mM Tris-HCl (pH 8.0), 25 mM EDTA (pH 8.0), and 0.1 mg/ml proteinase K. The lysates were then extracted once with phenol-chloroform-isoamyl alcohol (25:24:1) and twice with chloroform-isoamyl alcohol (24:1), followed by ethanol precipitation of the DNA with 2.5 M ammonium acetate. The DNA was then collected by centrifugation at 4000 rpm for 30 min, in a Beckman J-6 centrifuge. The pellets were washed with 70% ethanol, air-dried, and dissolved in TE buffer (10 mM Tris-HCl, 5 mM EDTA, pH 8.0). Spectrophotometric readings at 260 nm and 280 nm were used to determine both the concentration and quality of the DNA (30).

Detection of DNA Fragmentation. Eight μg of each DNA sample were incubated at 37°C for 1 h with 0.1 units of DNase-free RNase, to remove any residual RNA. The samples were then electrophoresed through a 2.0% agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.5) at 4 V/cm for 7 h. The DNA was then stained with ethidium bromide (0.5 μg/ml) for 30 min, followed by destaining in distilled water for 1 h. The DNA was visualized under UV light and photographed with Polaroid 665 film.

Southern Blot Analysis. DNA samples (100 μg) were digested with *Eco*RI (3.5 units/μg DNA) for 5 h. The reactions were terminated by phenol extraction, with subsequent ethanol precipitation. The DNA was dissolved in TE buffer (pH 8.0) and requantitated. The restricted DNA (10 μg) was then size-fractionated by electrophoresis through 1.2% agarose gels, in TAE buffer, for 16 h at 1 V/cm. The DNA was depurinated and denatured in preparation for transfer, as described by Wahl *et al.* (31). Transfer of the DNA to Biotrans nylon membranes (ICN) in 20× SSC (3 M sodium chloride, 0.3 M sodium citrate, pH 7.0) for 16–24 h was performed according to the method of Southern (32). Immobilization of the DNA was accomplished by drying the membranes in an 80°C oven for 2–5 h.

The membranes were prehybridized for 8 h at 42°C in a buffer containing 50% formamide, 5× SSC, 5× Denhardt's solution, 200 μg/ml sheared and denatured salmon sperm DNA, and 0.5% SDS. Probes were radioactively labeled by nick translation (33), heat-denatured for 5 min, and then added to the buffer (1.2×10^6 cpm/ml buffer), and hybridization was continued for 16–24 h (34). Consecutive washes were subsequently performed in 2×, 0.5×, and 0.1× SSC buffers containing 0.1% SDS, at room temperature for 15 min each, followed by a final

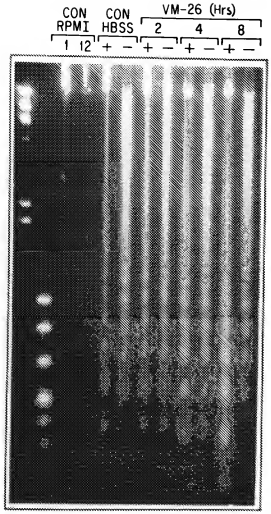

Fig. 2. Diminished DNA fragmentation caused by VM-26 in the absence of calcium. HL-60 (G_1) cells were cultured in RPMI 1640 medium as experimental controls (*CON*). Cells exposed to VM-26 (5 μM) were cultured for up to 8 h in calcium-sufficient (+) or -deficient (−) HBSS supplemented with 0.5% bovine serum albumin and 10 mM *N'*-[2-hydroxyethyl]piperazine-*N'*-2-ethanesulfonic acid. EGTA (8 mM) was added to the calcium-deficient HBSS to chelate any remaining calcium ions. DNA was isolated and electrophoresed as previously described.

wash of the membranes in 0.1× SSC/0.1% SDS at 42°C for 30 min. The membranes were then wrapped in plastic and exposed to X-ray film at −80°C. When appropriate, nylon membranes were stripped of the hybridized probe by immersion in a solution of 50% formamide/10 mM sodium phosphate (pH 7.4) for 2 h at 65°C. Complete removal of the probe was verified by the absence of signals on autoradiograms. Quantitation of band intensities on autoradiograms was accomplished using a Shimadzu CS9000U dual-wavelength Flying-Spot Scanner. Several different membranes were used in each experiment and all were rehybridized to the 18S rRNA cDNA probe. This permitted normalization of the signal intensities obtained from the different gene probes.

RESULTS

Time Course of VM-26-induced DNA Degradation in HL-60 Cells. DNA was isolated from HL-60 promyelocytic leukemia cells continuously exposed to 5 μM VM-26 for 1, 2, 3, 4, 8, and 12 h. Electrophoresis through 2.0% agarose gels and subsequent ethidium bromide staining of the DNA allowed visualization of nucleosomal ladders, and, therefore, of DNA endonuclease activity, as early as 3 h into the treatment (Fig. 1). From this time point onwards, lower molecular weight DNA degradative products predominated in each sample.

Inhibition of protein synthesis has been shown to inhibit or delay apoptosis (35). At each time point, cells pretreated with 20 μg/ml CHX, followed by exposure to VM-26 in the presence of CHX, exhibited less DNA fragmentation than the cells treated with VM-26 alone (Fig. 1). CHX treatment alone also resulted in a small amount of DNA degradation, which probably contributed to that seen in the VM-26 plus CHX combination. Depletion, or chelation, of intracellular and extracellular calcium can also retard the onset of apoptosis, due to inhibition of the Ca^{2+}/Mg^{2+}-dependent endonuclease. As shown in Fig. 2, the ratio of high molecular weight DNA to low molecular weight ladders was higher under calcium-free conditions than in the presence of calcium, implying a reduced DNA endonucleolytic activity. DNA degradation induced by high concentrations of VM-26 is thus rapid and is due to the induction of apoptosis.

Degradation of Transcribed Genes in HL-60 Cells Exposed to VM-26. The degradation of individual genes during apoptosis was examined by Southern blot analysis of *Eco*RI-digested DNA samples obtained from HL-60 cell cultures exposed to VM-26 (5 μM) for durations of up to 12 h. DNA transferred to nylon membranes was hybridized with radioactively labeled probes for the genes of interest, followed by autoradiography. The relative intensities of the resulting signals were determined by densitometric scanning of the films, and the percentage of intact gene DNA remaining at each time point was then calculated and tabulated (Table 1).

The amplified 18S rRNA and single-copy G6PD genes were chosen for studying the VM-26-induced degradation of "housekeeping" genes. Both are constitutively expressed under normal circumstances and have been frequently used as reference genes in Northern blot analysis because of this attribute. These genes were degraded with similar kinetics, with the highest rate of DNA degradation being apparent at 3 h after the beginning of VM-26 exposure, shown for the 18S rRNA gene in Fig. 3 and summarized in Table 1. The uniform nature of DNA degradation induced by teniposide is also illustrated by the identical rate of degradation of the two DNA bands produced by *Eco*RI cleavage of the 18S rRNA gene (Fig. 3).

Damage to several transcriptionally active and growth-regulated genes (*i.e.*, c-*myc*, c-Ha-*ras*, and *bcl*-2) was also examined. The c-*myc* proto-oncogene is amplified 20–30-fold and is highly expressed in HL-60 cells. C-Ha-*ras* and *bcl*-2 are both single-copy proto-oncogenes and have moderate to low levels of expression, respectively, in these cells. A significant decrease in c-*myc* gene DNA integrity occurred after 3 h of drug exposure (Table 1). This gene continued to be degraded, and at 12 h after addition of teniposide only 9% of the DNA encoding c-*myc* sequences remained intact. Although the copy number and transcriptional activities of c-Ha-*ras* and *bcl*-2 genes differed, both were degraded at an approximately similar rate as the c-*myc* oncogene (Table 1).

Transcriptionally Silent Genes and Expressed Genes Are Degraded Similarly during Apoptosis. The C_λ and J_H genes were used to determine the extent of endonuclease digestion of transcriptionally inactive genes during apoptosis, since these genes are never expressed in the myelomonocytic HL-60 cells. No difference in the rate or the extent of DNA degradation after 12 h of exposure to VM-26 was found between these genes

Table 1 *VM-26-induced degradation of selected DNA sequences*

DNA was extracted from HL-60 cells treated with VM-26 (5 μM) for the indicated periods of time, digested with *Eco*RI, and subjected to Southern analysis. The Southern blots were hybridized to probes for the indicated genes and DNA sequences. The resulting bands on the autoradiograms were quantitated densitometrically. All membranes were rehybridized to the 18S rRNA cDNA probe, and the sum of the densitometric values for the two mRNA gene bands was used for correction for transfer efficiency, prior to further calculations. Values represent the mean ± SD of three experiments, expressed as percentage of the intact DNA remaining at each time period, compared to that of the untreated control (0 h).

	Degradation (% of intact DNA remaining)						
Gene	0 h[a]	1 h	2 h	3 h	4 h	8 h	12 h
18S rRNA	100.0	94.9 ± 7.3	95.5 ± 11.4	55.3 ± 19.8	31.7 ± 17.2	22.2 ± 10.0	8.4 ± 4.3
G6PD	100.0	118.4 ± 10.6	119.2 ± 15.3	46.9 ± 9.5	22.8 ± 4.6	14.2 ± 8.6	5.2 ± 3.4
c-*myc*	100.0	76.8 ± 16.2	59.2 ± 16.2	41.8 ± 19.1	22.2 ± 11.1	14.6 ± 10.3	8.9 ± 6.3
c-Ha-*ras*	100.0	69.2 ± 11.7	73.6 ± 9.0	48.9 ± 12.6	25.6 ± 13.9	13.7 ± 7.4	7.6 ± 1.9
bcl-2	100.0	97.4 ± 9.2	98.2 ± 15.6	56.4 ± 19.7	30.1 ± 19.2	22.9 ± 11.9	22.6 ± 7.4
C_λ	100.0	87.2 ± 2.9	94.2 ± 26.5	59.5 ± 23.9	32.3 ± 17.6	22.7 ± 8.5	14.0 ± 4.0
J_H	100.0	73.5 ± 11.3	84.8 ± 19.1	58.2 ± 16.7	29.7 ± 0.9	21.1 ± 5.3	13.9 ± 3.8
mtDNA[b]	100.0	102.9 ± 14.5	137.0 ± 36.9	158.6 ± 51.7	123.6 ± 10.2	127.6 ± 20.5	142.0 ± 2.5

[a] Duration of VM-26 exposure.
[b] Determined with the p72 probe.

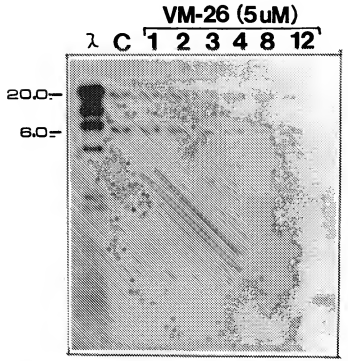

Fig. 3. Degradation of an active gene's DNA during continuous exposure to VM-26. HL-60 (G_1) cells were exposed continuously to VM-26 (5 μM) for periods of time up to 12 h. DNA was isolated, digested with *Eco*RI, and subjected to Southern blot analysis using the 18S rRNA cDNA probe, as described in "Materials and Methods." Untreated cells served as controls (*C*). Sizes of the hybridizing bands were determined using a *Hind*III-digested bacteriophage λ DNA molecular weight marker (λ).

and those previously discussed (Table 1). This is illustrated for the J_H gene in Fig. 4.

Resistance of Mitochondrial DNA to VM-26-induced DNA Degradation. The integrity of mtDNA during apoptosis was studied using a recently characterized mtDNA probe, p72, consisting of 600 base pairs of mtDNA encompassing the 3′-terminal portion of the mitochondrial 16S rRNA gene, the entire $tRNA^{Leu(UUR)}$ gene, and the 5′ end of the NADH dehydrogenase subunit 1 gene (27). As previously reported by us, hybridization of Southern blots of *Eco*RI-digested DNA with this probe yields an 8-kilobase band on autoradiograms (Fig. 5). Since the mitochondrial genome was isolated along with the nuclear DNA during DNA extraction, simultaneous analysis of mtDNA was possible. As shown in Fig. 5 and Table 1, mtDNA did not appear to be degraded at the times of VM-26 treatment which resulted in degradation of DNA in the nucleus. On the contrary, samples of total cellular DNA were found to be enriched for mitochondrial DNA at the later time points of VM-26 treatment, since nuclear DNA was degraded and lost from the sample applied to the gel (Table 1).

Inhibition of VM-26-induced Degradation of Nuclear Genes but not of the Mitochondrial Genome by Cycloheximide. The ability of CHX to delay teniposide-induced fragmentation of total cellular DNA was displayed above (Fig. 1). In order to determine if nuclear and mitochondrial DNA are affected differently under these conditions, the effect of CHX on VM-26-induced degradation of a nuclear gene (c-*myc* proto-oncogene) and mitochondrial DNA was compared (Fig. 6). Exposure of HL-60 cells to CHX alone for 12 h slightly decreased c-*myc* signal intensity on the Southern blot, possibly due to stimulation of DNA endonuclease activity. CHX given together with VM-26 markedly inhibited degradation of the c-*myc* gene DNA (Fig. 6*A*). In contrast, an enrichment for mitochondrial DNA was seen in DNA samples from cells treated with VM-26 alone for 8 or 12 h, and this enrichment was not apparent when cells were exposed to VM-26 and cycloheximide simultaneously (Fig. 6*B*). Indeed, a small decrease in band intensity, similar to that seen when c-*myc* was studied, was observed during combined treatment with VM-26 and CHX, showing that when CHX is present there is no difference in VM-26-induced degradation between nuclear and mitochondrial DNA.

DISCUSSION

Molecular biology of the cancer cell has been the subject of numerous investigations, and the basis for the abnormal growth properties and the abnormal behavior of the neoplastic cell is now understood, at least in broad terms. It appears that an overactivity, or an inappropriate activity, of proto-oncogenes or tumor suppressor genes upsets the delicate balance of the patterns of gene expression that regulate growth and differentiation of mammalian cells (36, 37). Surprisingly, therefore, knowledge of the mode of action of the chemical compounds which cause cell death and are effective in cancer chemotherapy has not kept pace with this progress. Useful drugs have arrived on the scene largely due to serendipity or mass screening of numerous natural products or synthetic organic chemicals. It was, therefore, very exciting when epipodophyllotoxin derivatives with significant antitumor activity, etoposide and teniposide, were found to be selective inhibitors of topo II activity, an

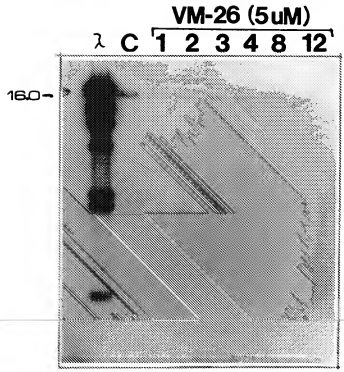

Fig. 4. VM-26-induced disappearance of the transcriptionally inactive J_H gene.

enzyme which participates in DNA replication and probably also transcription (6, 13, 38, 39). It was reasonably assumed that DNA damage produced by an interference with the activity of this enzyme is responsible for cell death (40). Several recent reports presented data which throw doubt on this assumption. For instance, topoisomerase II-targeting drugs which do not induce cleavable complex formation, such as merbarone, aclarubicin, and fostriecin, have cell-killing activity and can serve as antitumor agents (41–43). This suggests that enzyme-induced DNA cleavage is not necessarily the mechanism by which cells are killed by topoisomerase II-inhibiting drugs. Indeed, a dissociation between etoposide-induced DNA strand breaks and cytotoxicity has been reported (44). Overall, however, data that have accumulated favor the view that DNA strand scission is the initial event in teniposide and etoposide cytotoxicity, followed by a complex and poorly understood series of events which leads to cell death (9, 17, 45). An important parameter of drug action that kills mammalian cells is the concentration of the drug used. Above a certain threshold concentration for each topoisomerase II inhibitor, endonucleolytic activity may be triggered, producing a rapid degradation of nuclear DNA (46). This degradation affects principally the S and G_2 phase cells (47).

The results of the experiments presented here support this view. Cleavable complex formation is unlikely to degrade internucleosomal DNA preferentially. The production of the ladders by teniposide in these experiments is inhibited by a reduction in calcium concentration and addition of EGTA (Fig. 2), consistent with an activity of the known Ca^{2+}/Mg^{2+}-dependent nuclear DNA endonuclease (20). Furthermore, the appearance of nucleosomal ladders is delayed by the simultaneous addition of CHX, another feature of DNA endonuclease-mediated apoptosis (35). Whether the DNA endonuclease is newly synthesized under these conditions is controversial, since high concentrations of CHX given alone also produce nucleosomal ladders (17), but protein synthesis appears to accelerate the endonucleolytic cleavage when intermediate concentrations of teniposide are used, as in the experiments described here.

The resistance of mitochondrial DNA to teniposide also suggests that the formation of a cleavable complex is insufficient to result in DNA degradation. Data in the literature indicate that teniposide penetrates into mammalian mitochondria (48) and that topoisomerase II is present in the mitochondria of human leukemic cells (49). The mitochondrial topoisomerase II is inhibited by teniposide in a manner identical to the inhibition of nuclear topoisomerase II (50). This again points to the importance of a DNA endonuclease in DNA degradation that follows exposure to teniposide. We propose that the cleavable complex formation initiates a chain of events which rapidly leads to activation of a calcium-activated nuclear or cytoplasmic endonuclease and involves a process facilitated by protein synthesis.

The nuclear genes examined were degraded at rates which were indistinguishable whether the gene was regulated, constitutively expressed, or never transcribed in these cells. Transcribability of the gene is, therefore, not a factor in its degradation following exposure to teniposide. Since topoisomerase II is thought to be involved in transcription (6, 13, 39), our data suggest that either its local density on DNA is not determined by transcription or the cleavable complex formation has no direct effect on DNA degradation.

Self-destruction of a cell is a necessary part of the organism's development and adaptation to injury and has been shown to be, at least in part, an active process (19, 35). The differential sensitivity of nuclear and mitochondrial DNA to the degradative intracellular forces is an interesting facet of the complex events that lead to cell death. The role of a DNA endonuclease in these processes seems to be clear, but the details of its activation following cleavable complex formation remain to be elucidated.

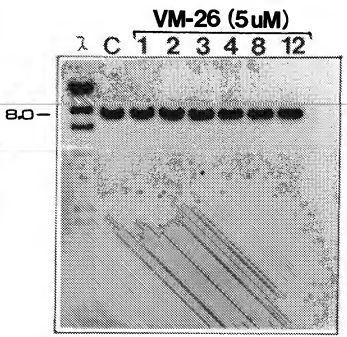

Fig. 5. Resistance of mitochondrial DNA to VM-26-induced degradation. The nylon membrane probed for the 18S rRNA gene in Fig. 3 was stripped of radioactivity and rehybridized with the ^{32}P-labeled p72 mtDNA probe.

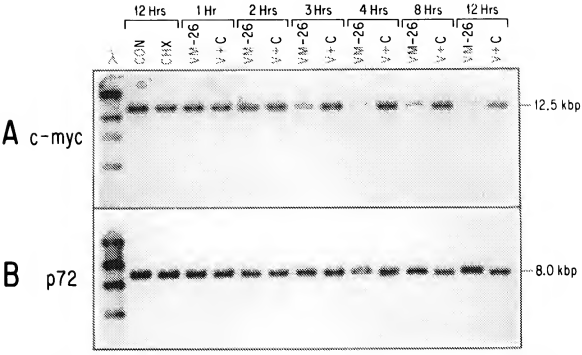

Fig. 6. Inhibition of *c-myc* gene DNA degradation by cycloheximide. HL-60 (G_1) cells were exposed to VM-26 (5 μM) for 1–12 h or pretreated with CHX (20 μg/ml) for 4 h, followed by similar treatment with VM-26 (*V+C*). Untreated (*CON*) and CHX-treated (*CHX*) cells were used as controls. Southern blot analysis of the DNA was performed after *Eco*RI digestion. The membrane was first probed for the presence of *c-myc* gene DNA using the pRyc 7.4 plasmid (*A*), followed by stripping and rehybridization with the p72 mitochondrial DNA probe (*B*).

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